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FINAL REPORT

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ADAPTATIVE RESPONSE OF SLOW AND FAST SKELETAL MUSCLE IN THE MONKEY TO SPACEFLIGHT

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The proposed experiments were designed to determine the effects of the absence of weight support on hindlimb muscles of the monkey: an ankle flexor (tibialis anterior, TA), two ankle extensors (medial gastrocnemius, MG and soleus, SOL), and a knee extensor (vastus lateralis, VL). These experiments will be performed as part of the BION mission in collaboration with Dr. Reggie Edgerton at UCLA.

The original project proposed to assess the effects of weightlessness in adult Rhesus monkeys which were to be flown on the Space Shuttle as part of SLS-3. Feasibility studies were carried out and a series of experiments were performed at NASA/Ames Research Center to assess the effects of a 21-day restraint period in the ESOP on muscle properties. The results of these studies will be summarized.

HYPOTHESES

Muscle fibers will atrophy when subjected to a 0 gravity environment. Based on previous data obtained in rats after a 5-14 day spaceflight and after hindlimb suspension, we hypothesize that muscle fibers in a slow muscle will atrophy more rapidly than fibers in a fast muscle, and slow fibers in either a predominantly slow or mixed muscle will atrophy more than fast fibers.

The normal oxidative and glycolytic potential of muscle fibers will be sustained during the rapid phases of muscle atrophy while myosin isozymes of some fibers will change from slow toward fast types.

OBJECTIVE

Define the degree of muscle atrophy and metabolic changes that occur in muscle fibers of Rhesus following 14 days of microgravity.

METHODS

In preparation for the proposed SLS-3 flight we participated in 3 separate ground-based experiments in which adult male Rhesus monkeys were placed in the ESOP for a maximum of 21-days. Table 1 gives a summary of the monkeys that were biopsied prior to (PRE) and immediately after (POST) the restraint period.

Muscle biopsies were obtained 2-weeks to 8-weeks prior to the testing period (pre) and 12 hours after (post) a 21 day restraint period in the ESOP. The control monkeys remained in their individual cages during the 21-day restraint. Biopsies were taken from two independent sites in the soleus (Sol), medial gastrocnemius (MG), and tibialis anterior (TA) muscles using an open biopsy technique. All biopsies were taken from the right leg.

The biopsies were taken while the animal was under a general anesthesia (inhalation of isoflurane). Under sterile conditions, small incisions (3-4 cm) was made on the medial side of the lower leg to expose the Sol and MG muscles. Using blunt dissection, the belly of each muscle was exposed and a small cut was made in the overlying fascia. To obtain the biopsies, the tip of a scalpel blade was used to isolate a piece of tissue approximately 10 mm long x 5 mm wide x 5 mm deep (~150 mg wet weight). All samples were taken from the superficial muscle belly and the cut was made parallel to the direction of the muscle fibers. The muscle sample was removed and immediately placed on a saline soaked guaze.

The fascia and skin were closed with absorbable sutures (Vicryl ™). The tissue sample was weighed, stretched to approximately the in situ length, and mounted on cork using pins to insure a perpendicular orientation of the muscle fibers. The samples were frozen in isopentane cooled with liquid nitrogen and stored at -80° C. The pre restraint biopsies were taken from the medial regions of the proximal Sol and the distal MG. The post restraint biopsies were taken from the medial regions of the distal Sol and and the middle region of the proximal MG.

The biopsy sites were selected to ensure that the same muscle fibers were not sampled during the pre and postflight biopsies and were determined based on detailed architectural analyses of each muscle (Roy et al., 1991). In addition, the feasibility of obtaining reproducible biopsies from the selected sites has been verified, i.e., the regions sampled within each muscle have been shown to have similar fiber type distributions (Roy et al, 1991).

All surgeries were performed at NASA/ARC. The biopsy samples have ranged in size from 150 to 300 mg. The biopsy samples taken from the SOL and MG were shared with Dr. Robert Fitts.

TISSUE ANALYSIS

Fiber Cross-Sectional Area: The mean fiber cross-sectional area of each biopsy sample was determined from a population of 100 to 400 fibers measured from a serial cross-section immunohistochemically stained with a monoclonal antibody for laminin. A Vectastain ABC™ kit (Vector Labs, Burlingame, CA, USA) was used to amplify the antigen-antibody complex, which in turn, was visualized by treatment with a diaminobenzidine (DAB) peroxidase reaction. Laminin stains the basal lamina just outside the plasma membrane (26). Using a color camera attached to a light microscope and image analysis system (Image I-AT, Universal Imaging Corporation), the region within the laminin boundaries was automatically filled in by the computer and the fiber cross-sectional area was calculated in μm^2.

Succinate dehydrogenase Activity : Succinate dehydrogenase (SDH) activity and fiber cross-sectional area were determined for individual fibers (50-90 fibers) in 10 µm cross sections taken from each of the biopsy samples. Tissue sections were analyzed on a computer-assisted image analysis system (Image-I/AT, Universal Imaging Corporation). To measure SDH activity, repeated digitized images were taken of a single tissue section every 2 min over a period of 14 min while the tissue was incubated in a medium without the substrate, succinate. A medium with succinate then was added and repeated scans were taken every 2 min over the next 14 min. Reaction rates for each fiber were based on a linear regression line determined from the 8 points acquired with the medium containing substrate. Although the absolute optical density readings may vary slightly from day to day, the slope or reaction rate is not affected.

The medium for determining SDH activity contained: 100 mM phosphate buffer (pH=7.6), 1.5 mM sodium azide, 3 mM 1-methoxyphenazine methylsulfate, 1.5 mM nitro blue tetrazolium, 5.5 mM EDTA-disodium salt, and 58 mM succinate disodium salt. This incubation medium is a modification of the one used for the rat muscle and has been optimized for the monkey muscle. The reaction rates for fibers in the monkey are approximately 5 times slower than those in the rat.

Fiber Types: Fibers were classified as type I, IIa, IIb, IIx or hybrid (coexpression of slow and fast) using monoclonal antibodies which label the different myosin heavy chain

(MHC) isoforms. Serial cross-sections were incubated with primary antibodies (BA-F8, BF-13, BF-35 and SC-71 generously donated by S. Schiaffino (Padova, Italy)) overnight at 25°C. Sections incubated without primary antibody were used as controls to visualize non-specific labeling. A Vectastain ABC™ kit (Vector Labs, Burlingame, CA, USA) was used to amplify the antigen-antibody complex, which in turn, was visualized by treatment with a DAB peroxidase reaction.

The immunohistochemical labeling was compared to standard histochemical staining for myofibrillar ATPase after acid (pH, 4.35) and alkaline (pH, 10.0) preincubation. Serial sections also were stained for hemotoxylin and eosin (H&E) for routine histological examination.

SUMMARY OF RESULTS

Fiber Cross-Sectional Area:

There was a significant decrease in the mean fiber size in the Soleus following a 21-day restraint in the ESOP (Figure 1). There was no significant change in mean fiber size in the MG or TA. Individual pre and post restraint data is provided in Figure 2. In the 4 control monkeys studied, fiber size increased in all three muscles (Sol, MG and TA) between the pre and post restraint samples. In the experimental monkeys, Sol fiber size decreased in 7 of the 7 monkeys studied; MG fiber size decreased in 3 of 7 and increased in 3 of 7 monkeys; TA fiber size increased in 3 of 4 and decreased in 1 of 4 monkeys.

Oxidative Potential:

SDH activity did not significantly change following the 21 day restraint period. The relationship between fiber size, SDH activity and fiber type is shown in Figure 3. In general, the type IIx fibers are the largest in size and have the lowest SDH activity. Slow fibers in the Sol are larger than slow fibers in the MG or TA.

Fiber Type

Fibers were classified as type I, IIa, IIx or hybrid (coexpression of slow and fast) using monoclonal antibodies which label the different myosin heavy chain (MHC) isoforms.

Muscle fibers from the Sol and MG could be classified into four types based on their immunohistochemical staining to monoclonal antibodies to the myosin heavy chain. The type I fibers were positive for the BA-F8 (only slow MHC) and BF-35 (all MHCs except IIx) antibodies, and negative for the BF-13 (all type II MHCs) and SC-71 (only IIa MHC) antibodies. The type IIa fibers were positive for all of the antibodies except the F-8 antibody which is specific for the slow MHC. The classification of IIx was based on the negative staining of fibers for the BF-35 antibody. These fibers were also negative for BA-F8, positive for BF-13 and intermediate for SC-71. In the rat, the SC-71 antibody is specific for type IIa fibers. In the monkey and humans (personnal communication, S. Schiaffino), however, the IIx fibers stain intermediate for SC-71. Based on these antibodies we could not detect any type IIb fibers. Hybrid fibers stained positively for all the antibodies and presumably expressed both type I and IIa MHCs.

The average fiber type distributions in the Sol, MG and TA are given in Figure 4. There was no change in the fiber type distributions following a 21-day restraint. Table 2 gives the average for the pre and post restraint samples taken from the Sol and MG. There was no significant difference between control and experimental samples.

SUMMARY

Restraint for a period of 21-days in the ESOP resulted in significant atrophy in the Soleus, but not the MG and TA. In a previous study we examined the effects of a 14-day restraint in the BIOS chair used in the Russian Biosatellite. Following a 14-day restraint in the BIOS chair we found no significant atrophy in the Sol, MG or TA. Consequently, the ESOP is a more restrictive environment than the BIOS chair. We foresee no difficulties in evaluating the data to be obtained from the upcoming BION missions.

Based on data obtained from rats flown in space, the initial hypothesis stated that extensor muscles such as the soleus (Sol) and medial gastrocnemius (MG) would atrophy more than flexor muscles such as the tibialis anterior (TA). However, data obtained on Cosmos 2044 and 2229 showed that the TA atrophied to a greater extent than the Sol and MG. In Cosmos 2044, both the Sol and the MG were larger postflight than preflight. These data suggested that the monkeys were doing some type of "exercise" in flight. Consequently, we have revised our original hypothesis. We now believe that the Sol and MG are spared from the effects of microgravity due to the ability of the monkeys to apply external loads to the fixed foot pedal while restrained in the BIOS chair.

Participation in BION 11 and 12 will enable us to increase our sample size from 4 to 8 monkeys. The data from Cosmos 2044 and 2229 suggests that the flexors atrophied to a greater extent than the extensors. This is contrary to what has been reported in the rat following spaceflight and hindlimb suspension. One explanation for this finding is that the restrained monkeys are performing some type of exercise while in space. In BION 11 and 12 this hypothesis could be tested by monitoring the activity and forces generated by the "non-trained" right limb.

In the previous flights we have measured fiber type conversion with monoclonal antibodies. In BION 11 and 12 we will continue to use immunohistochemistry to study protein changes, but we will add an analysis of myosin heavy chain (MHC) mRNA expression. We have recently cloned 700 bases of the 3' region of the MHC mRNA in both rats and humans. We have recently tested the human mRNA sequences on monkey tissues and have gotten positive results. Consequently, in BION 11 and 12 we will study both MHC protein and mRNA expression.

PUBLICATIONS

The data collected from the ground-based testing on the adult Rhesus monkeys will be submitted in two manuscripts. The first manuscript will be written in collaboration with Dr. Fitts and will discuss the effects of the restraint on hindlimb muscles. The second manuscript will describe the properties of adult primate muscles. I will also compare the data collected from the adult monkeys with that collected on the juvenile monkeys used in the Cosmos project.

TABLE 1

SUMMARY OF GROUND BASE EXPE RIMENTS AT NASA/ARC

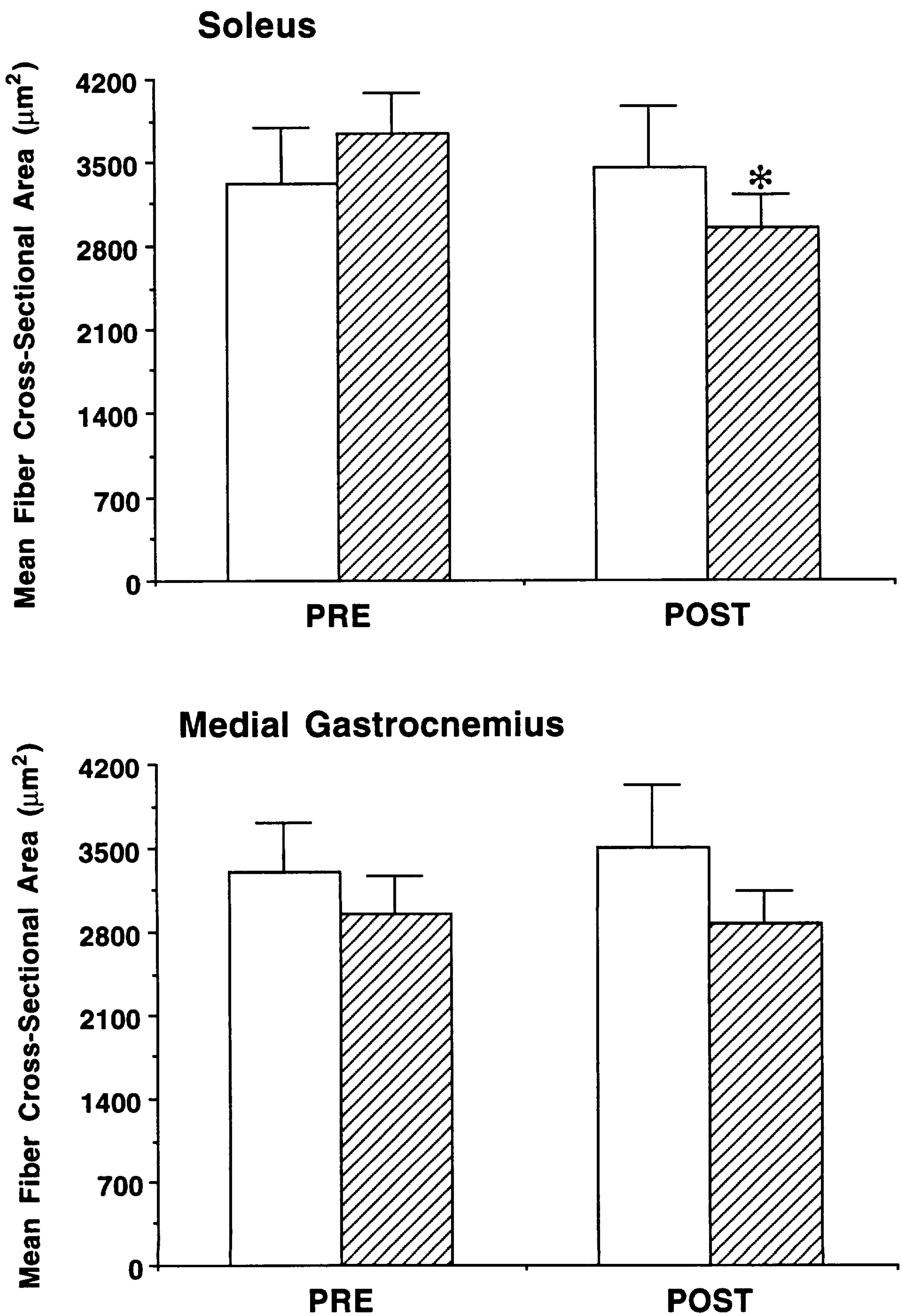
MONKEY	GROUP	BIOPSY	DATE	BODY WEIGHT (kg)
738	RESTRAINT	PRE	5-22-92	9.4
	(21-day ESOP)	POST	6-23-92	8.8
311	RESTRAINT	PRE	2-17-93	7.6
(11)	(21-day ESOP)	POST	5-4-93	8.6
288	RESTRAINT	PRE	2-17-93	9.2
(10)	(21-day ESOP)	POST	5-4-93	9.3
81-242	CONTROL	PRE	2-18-93	9.5
(1)		POST	5-5-93	9.6
E-613	CONTROL	PRE	2-18-93	10.2
		POST	2-5-93	10.4
85-312	**RESTRAINT**	PRE	9-8-93	10.9
(7)	(SIT 1)	POST	11-29-93	10.8
87-164	**RESTRAINT**	PRE	9-8-93	9.3
(5))	(SIT 1)	POST	11-29-93	9.2
83-337	CONTROL	PRE	9-9-93	9.6
(3)		POST	11-30-93	11.1
H-593	CONTROL	PRE	4-21-94	10.8
(4)		POST	6-16-94	11.2
84-318	CONTROL	PRE	4-20-94	10.2
(2)		POST	6-16-94	12.4
85-309	**RESTRAINT**	PRE	4-19-94	9.3
(6)	(SIT 2)	POST	6-17-94	9.3
H-534	**RESTRAINT**	PRE	4-19-94	9.0
(8)	(SIT 2)	POST	6-17-94	9.5
H-602	**RESTRAINT**	PRE	4-20-94	11.0
(9)	(SIT 2)	POST	6-18-94	11.2

Table 2

Fiber Type Distributions in the Soleus and Medial Gastrocnemius

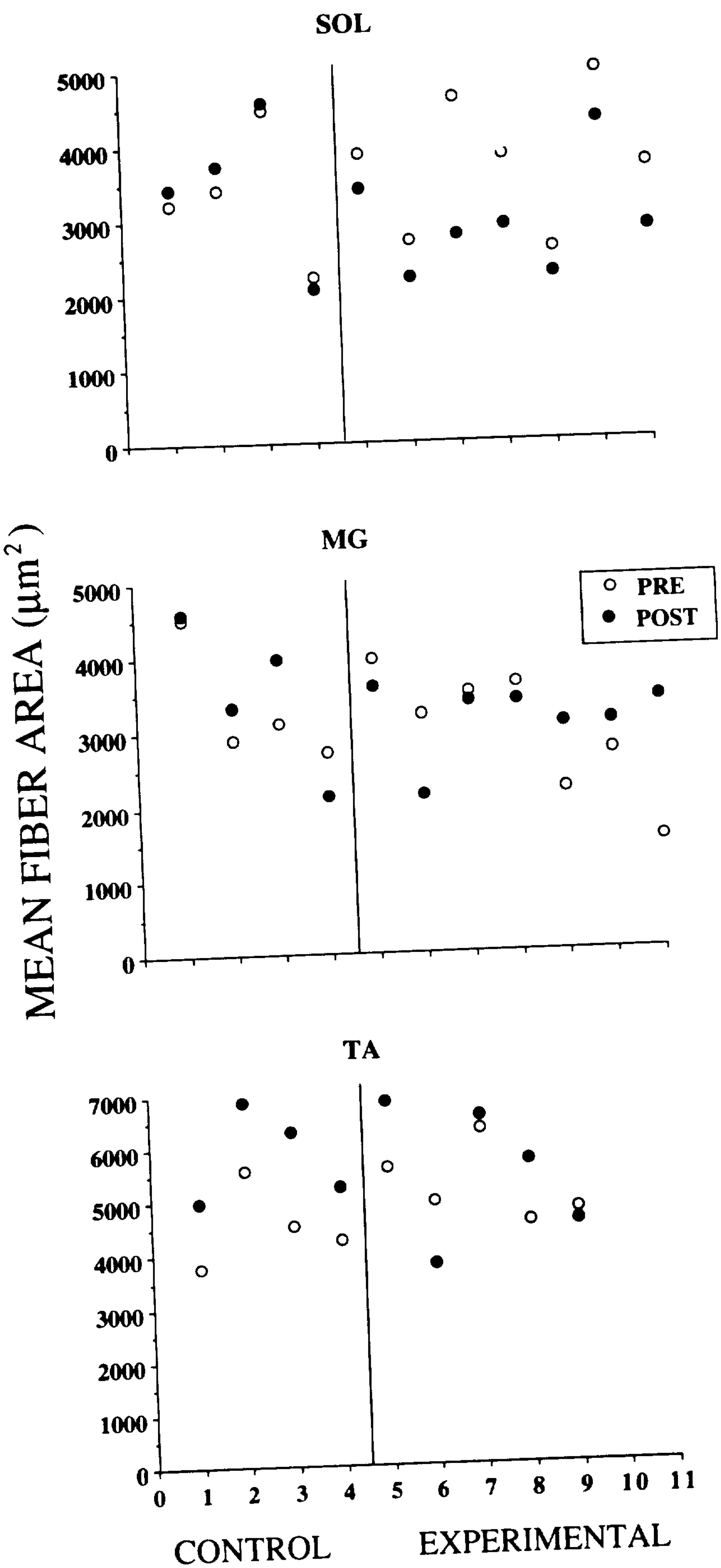
SOL		I	IIa	IIx	Hybrid
CON	PRE	79 ±10	12 ±5	0	9 ±4
	POST	62 ±14	21 ±9	0	17 ±5
EXP	PRE	62 ±6	22 ±5	0	16 ±3
	POST	51 ±6	25 ±6	0	24 ±3
MG					
CON	PRE	23 ±2	24 ±6	49 ±3	4 ±3
	POST	19 ±15	26 ±4	51 ±5	4 ±3
EXP	PRE	20 ±6	27 ±4	49 ±7	4 ±2
	POST	17 ±3	28 ±5	51 ±7	4 ±3

Data are mean ± standard error. Sample size was 4 (control) and 7 (experimental). An average of 403 ± 130 (SD) fibers were sampled in each biopsy.



Soleus
Mean Fiber Cross-Sectional Area (μm^2)
4200
3500
2800
2100
1400
700
0
*
PRE
POST
Medial Gastrocnemius
Mean Fiber Cross-Sectional Area (μm^2)
4200
3500
2800
2100
1400
700
0
PRE
POST

Figure 1



SOL
MG
TA
MEAN FIBER AREA (μm²)
PRE
POST
5000
4000
3000
2000
1000
0
7000
6000
0 1 2 3 4 5 6 7 8 9 10 11
CONTROL
EXPERIMENTAL

Figure 2

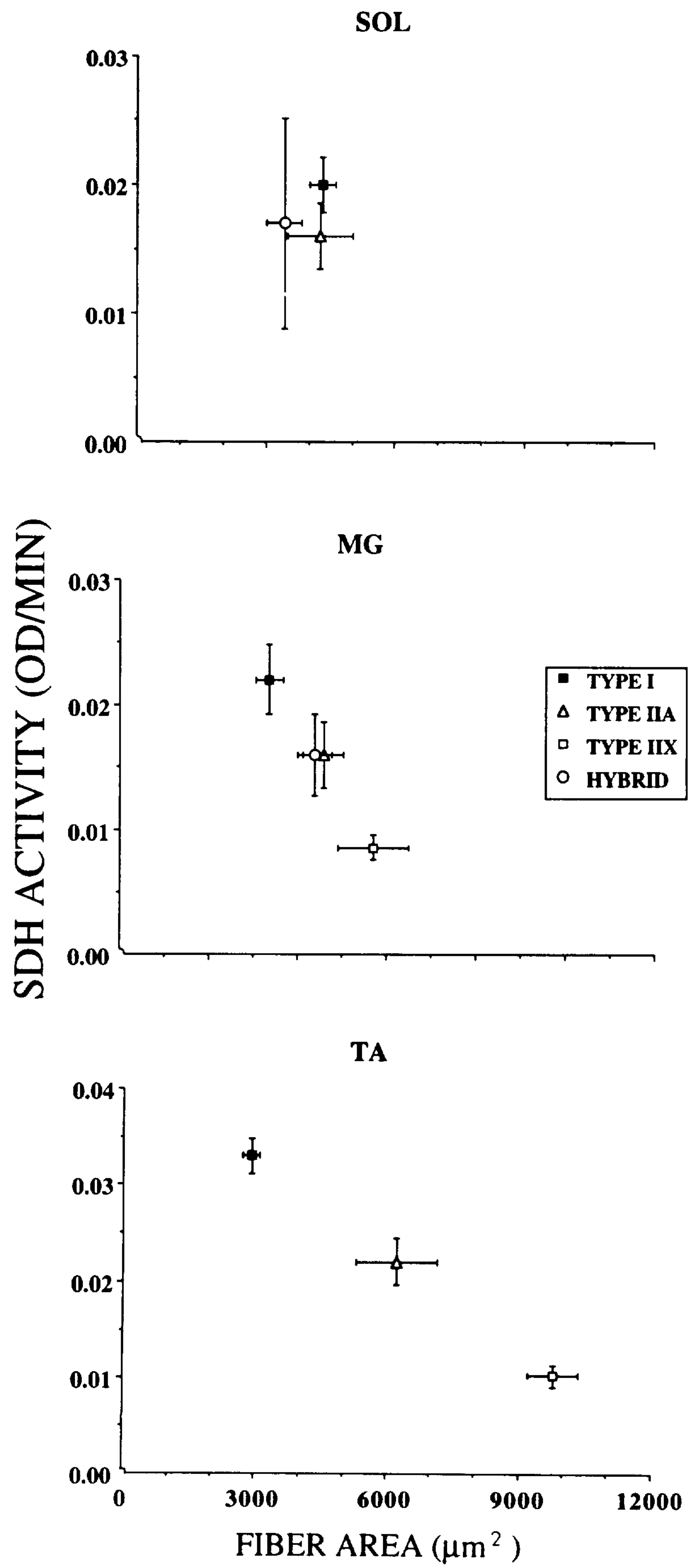

SOL
MG
TA
SDH ACTIVITY (OD/MIN)
FIBER AREA (μm²)
TYPE I
TYPE IIA
TYPE IIX
HYBRID
0.00
0.01
0.02
0.03
0.04
0
3000
6000
9000
12000

Figure 3

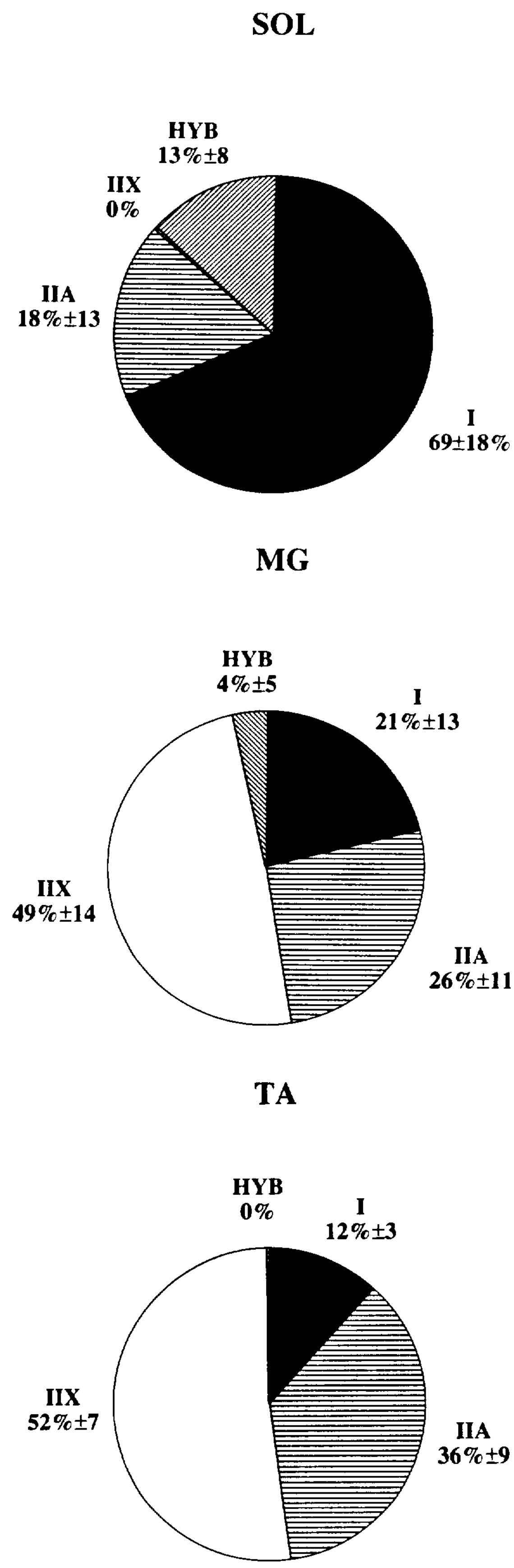

SOL
HYB
13%±8
IIX
0%
IIA
18%±13
I
69±18%
MG
HYB
4%±5
I
21%±13
IIX
49%±14
IIA
26%±11
TA
HYB
0%
I
12%±3
IIX
52%±7
IIA
36%±9

Figure 4